



Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at <http://about.jstor.org/participate-jstor/individuals/early-journal-content>.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

AN EXPERIMENTAL STUDY OF THE INFLUENCE OF
IODIN AND IODIDS ON THE ABSORPTION OF
GRANULATION TISSUE AND FAT-FREE
TUBERCLE BACILLI

STUDIES ON THE BIOCHEMISTRY AND CHEMOTHERAPY OF
TUBERCULOSIS. X*

EDWIN FREDERICK HIRSCH

(From the Pathological Laboratory of the University of Chicago and the Otho S. A. Sprague Memorial Institute.)

Cantacuzene,¹ in 1905, reporting an experimental study of the effects of fat-free tubercle bacilli on guinea-pigs, records that the bacilli lose much of their toxicity if treated with an iodine solution (I = 1 gm.; KI = 2 gm.; water = 400 c.c.) previous to their inoculation, and that they are then absorbed much faster than non-iodized bacilli. The resorption of fat-free bacilli and of tuberculous formations in guinea-pigs, he continues, is greatly enhanced by the daily administration of potassium iodide, the iodine salt markedly stimulating the phagocytic property of the endothelial leukocytes.

Cantacuzene's observations on the influence of iodides upon fat-free tubercle bacilli stand alone in the literature. They are especially important on account of the use of iodine and iodides in tuberculosis and in conditions where the absorption of granulation and necrotic tissue is desired. Because of this important bearing, a repetition of Cantacuzene's experiments seemed desirable, together with experiments in which agar was used to cause the formation of nodules of granulation tissue.

The human tubercle bacilli used in these experiments were obtained from Parke, Davis and Company and prepared by Dr. H. J. Corper of this laboratory. They were washed repeatedly with normal salt solution and dried. The dried bacilli were extracted in a Wiley extraction apparatus with several changes of hot absolute alcohol and several changes of warm ether, dried, pulverized in a malt mill, and again extracted with hot absolute alcohol and warm ether. The minimal lethal dose of the dried, fat-free bacilli, determined by the intraperitoneal inoculation of guinea-pigs, is about 0.04 gm. for each 100 gm. of guinea-pig weight. The agar jelly was melted and injected into the animals with a

* Received for publication August 21, 1914.

1. Ann. de l'Inst. Pasteur, 1905, 19, p. 699; Compt. rend. Soc. de biol., 1905, 59, p. 314.

powerful paraffin syringe. All injections were made with strict aseptic precautions. The potassium iodid was given subcutaneously in 2 percent strength, this being a concentration that does not cause necrosis.

SERIES 1. CONTROLS

April 21. The guinea-pigs of this series each received 0.01 gm. of fat-free tubercle bacilli intraperitoneally.

1. Female, 450 gm., killed April 29. On the surface of the liver are two white nodules, smaller than a pin head. There are several similar nodules on the surface of the spleen and on the serous surfaces of the abdominal wall. There are many white nodules, pin head and less in diameter, in the omentum and mesentery, on the stomach, and on the inferior surface of the diaphragm.

The nodules on the liver contain bacterial masses varying in size from those just discernible with the low power to others occupying the entire center of the field. These masses are irregular in outline and surrounded by large numbers of polynuclear leukocytes outside of which is a concentric layer of epithelioid and connective tissue cells. Along the periphery there are small capillaries. A few giant cells are present in the granulation tissue.

2. Male, 470 gm., killed May 7. There are six or more white nodules, less than a pin head in size, on the upper and lower surfaces of the liver and on the spleen. In the mesentery and particularly in the omentum just below the stomach are nodules varying in size up to that of a small pea. These nodules are firm and have a rather irregularly serrated white margin. On the inferior surface of the diaphragm there are white nodules about a millimeter in diameter and on the anterior abdominal wall are somewhat larger nodules which are firmly embedded.

Sections from the nodules on the spleen, in the omentum, liver, and parietal wall have the following characteristics: They contain small, granular masses of the bacteria, irregular in size and outline; numerous polynucleated leukocytes lie around these masses, some of them with fragmented nuclei; about this aggregation of polynuclears are many large endothelial cells which, toward the periphery, have a concentric arrangement and in turn are surrounded by vascularized granulation tissue. There are small giant cells in the granulation tissue.

3. Killed May 12, 450 gm. There are small, pearly white nodules on the inferior surface of the diaphragm, on the peritoneum, in the omentum, and mesentery. There are larger nodules, 2 to 4 mm. in diameter, on the anterior abdominal wall. The spleen is mottled by nodules, 2 to 3 mm. and less in diameter.

The nodules in the omentum and mesentery consist largely of cellular elements. There are small circumscribed areas the centers of which contain granular masses of bacteria which in turn are surrounded by a large number of polynuclears and endothelial cells or epithelioid cells which toward the periphery have a concentric arrangement. The margins of the nodules consist of typical granulation tissue in which there are small capillaries. There are no well-formed giant cells in the sections studied.

4. Female, 400 gm., found dead May 20. On the parietal peritoneum are small white plaques of scar tissue. There are three or four white nodular scars on the spleen and a number on the ventral abdominal wall and inferior surface of the diaphragm. There is a single firm nodule, 3 to 4 mm. in diameter, in the omentum.

The nodules on the spleen consist chiefly of granulation tissue. In the center of rather sharply circumscribed round areas are irregular masses of bacteria surrounded by numerous polynuclears. Epithelioid and endothelial cells lie around this central portion, and, along the periphery of the areas, they assume a concentric arrangement. Vascularized granulation tissue marks the boundary of the areas, and constitutes the bulk of the intervening tissue. The nodules in the mesentery have much the same histological structure.

Summary—Unabsorbed bacterial masses and granulation tissue are present, 39 days after the inoculation of tubercle bacilli, in guinea-pigs receiving no iodids.

SERIES 2. THE EFFECT OF IODIZED FAT-FREE TUBERCLE BACILLI INJECTED
INTRAPERITONEALLY

April 21. Each guinea-pig received 0.01 gm. of fat-free tubercle bacilli intraperitoneally. Previous to injection, the bacilli were treated for 15 minutes with 10 c.c. of an iodine solution (I=1 gm.; KI=2 gm; water=400 c.c.). The bacilli were sedimented by centrifuging and then suspended in a sterile normal salt solution. Immediately following the inoculation the pigs gave evidence of marked abdominal irritation.

1. Male, 440 gm., found dead April 22. In the peritoneum is a large amount of clear, straw-colored fluid. On the surfaces of the liver are yellowish deposits of bacteria varying in diameter from 2 to 3 mm. to a pin point. There are similar nodules on the spleen, stomach, and scattered throughout the entire mesentery and omentum.

2. Male, 440 gm., killed May 7. There are only a few scars suggestive of nodules on the inferior surface of the diaphragm. There are dense fibrous adhesions between the liver and the stomach, and in the omentum. The parietal peritoneum is rough and irregular from fibrous scars. There are pearly white areas, several millimeters and smaller in size, from which irregular white bands of scar tissue radiate. There are a few distinct nodules in the mesentery, but they are rather soft.

The nodules on the liver are encapsulated by fibrous tissue; scattered through their interior are irregular masses of bacteria all surrounded by numerous polynuclear leukocytes. Just outside the leukocytes are many epithelial and connective-tissue cells and along the periphery are numerous budding capillaries. There are many small giant cells, which apparently have been formed by the fusion of endothelial cells, about the masses of bacteria.

3. Male, 450 gm., killed May 12. The peritoneal surfaces are roughened by fibrous tissue. There are dense fibrous adhesions between the intestinal loops as well as omental adhesions with the stomach, abdominal wall, and liver. There are only two to four distinct nodules on the peritoneum which have a diameter of from 5 to 8 mm. The surface of the spleen is mottled by white spots which are rather tough and fibrous from connective tissues. There is no free fluid in the peritoneal cavity.

The nodules are surrounded by a well-developed wall of connective tissue. In the interior of the nodules are numerous irregular granular masses of bacteria surrounded by many polynuclears. The leukocytes form the bulk of the cellular elements. There are also endothelial and small giant cells. In some places, small bacterial masses are engulfed by giant cells which apparently have

formed by the fusion of endothelial cells. Concentrically arranged epithelioid and connective-tissue cells border the polynuclear leukocytes, while throughout the nodules run septa of granulation tissue. The abundance of cellular elements and giant cells is very pronounced.

4. Male, 430 gm., killed June 12. There are dense fibrous adhesions between the anterior abdominal wall and the intestines, between the liver and diaphragm, and between the loops of the bowel. There are a few pin-head sized nodules in the mesentery. The omentum is bound down to the liver in such a way as to form a dense mass.

Microscopically, there is an abundance of cellular tissue in the nodules, especially giant cells. Many of the giant cells contain small masses of bacteria. Other larger masses of bacteria are surrounded by polynuclears. Fibrous granulation tissue is very abundant.

Summary—There are unabsorbed bacterial masses and granulation tissue present even after 52 days. The treatment of the bacteria with iodine solution had no apparent effect on the absorption.

SERIES 3. THE EFFECT OF DAILY INJECTIONS OF POTASSIUM IODIDE ON THE ABSORPTION OF FAT-FREE TUBERCLE BACILLI AND GRANULATION TISSUE

Guinea-pigs 1 to 4 of this series received 0.04 gm. of potassium iodide daily and Guinea-pigs 5 to 8, 0.1 gm., all being injected intraperitoneally at the same time, April 21, with 0.01 gm. of fat-free tubercle bacilli.

1. Male, 480 gm., killed April 29. White nodules, pin head and smaller, are scattered through the omentum and mesentery. They are also present on the inferior surface of the diaphragm, on the peritoneal surfaces, the spleen, stomach, and liver.

Microscopically, in the nodules are numerous irregular masses of bacteria surrounded by a large number of polynuclear leukocytes, which form a relatively wide zone. Bordering this zone is a narrow margin of epithelioid cells and a small amount of vascular connective tissue. There are no definite giant cells.

2. Male, 460 gm., killed May 7. There are millimeter to pin point sized white nodules on the liver, stomach, and inferior surface of the diaphragm. The omentum is densely packed with firm, yellowish, or grayish-white, nodules varying in size from 3 to 4 mm. to a pin point. The spleen is normal except from 8 to 10 white nodules, 0.5 mm. and less in diameter, on its surface. There are a few nodules on the anterior abdominal wall, and here there is a firm adhesion with the left seminal vesicle. Otherwise the peritoneal surfaces are smooth. In the mesentery there are a few small nodules of variable size.

The nodules contain many small bacterial masses which have irregular outlines and are surrounded by a large number of polynuclear leukocytes at the border of which are concentrically arranged epithelioid and connective-tissue cells. The nodules are fairly well encapsulated by loose vascular connective tissue. Throughout the nodules are trabeculae of young connective tissue. Giant cells are not numerous, there being only an occasional one present which is apparently formed by the fusion of endothelial cells.

3. Female, 420 gm., killed May 12. On the inferior surface of the diaphragm and on the liver are a few small, hard, white nodules. The omentum is adherent to the abdominal wall in the region of the spleen which is also

adherent to the diaphragm. The surface of the spleen is mottled with white areas and there are a few adhesions between the intestinal loops. There is a little scar tissue on the anterior abdominal wall.

The nodules, examined microscopically contain masses of bacteria irregular in size and shape, which are immediately surrounded by a dense layer of polynuclear leukocytes. Bordering these are concentrically arranged epithelioid cells. Small capillaries are present in the tissue. The periphery of the nodule is formed by a connective-tissue capsule with numerous capillary buds. There are also occasional giant cells with three to eight nuclei, which apparently have been formed by the fusion of endothelial cells about a small mass of bacteria, or in the close vicinity of one of the larger masses.

4. Male, 410 gm., killed May 27. There are small white scars on the inferior surface of the diaphragm. The surface of the liver is spotted with yellowish-white nodules up to 2 mm. in diameter. In the omentum are nodules, 2 to 3 mm. in diameter, and smaller pearly-white ones are in the mesentery. On the ventral wall of the abdomen are nodules surrounded by scar tissue. There are adhesions between the intestinal loops and also between the loops and the anterior abdominal wall.

Masses of bacteria, irregular in size and shape, are in the nodules removed from the viscera. These masses are surrounded by polynuclear leukocytes, bordered in turn by epithelioid and young connective-tissue cells in concentric arrangement. The margin of the nodules is formed by dense connective tissue in which are well-formed capillaries and small vessels. There is much connective tissue in the nodules and frequently smaller bacterial masses are surrounded by a narrow ring of protoplasm containing numerous elongated, vesicular nuclei at either pole. Typical giant cells are numerous in the nodules.

5. Male 400 gm., killed April 29. There are many nodules, 2 mm. and smaller in diameter, scattered on the omentum, on the peritoneal surfaces, in the inferior surfaces of the diaphragm, on the liver, and the spleen.

Large and small masses of bacteria, irregular in outline and surrounded by many polynucleated leukocytes, are present in the stained sections of the nodules in the viscera. There is a thin capsule of young connective tissue about the nodules from which trabecula extend inward and appear as interrupted septa within the nodules. There are numerous endothelial cells irregularly scattered among the polynucleated leukocytes. Giant cells are not present to any great extent.

6. Male, 450 gm., killed May 8. On the inferior surface of the diaphragm, on the stomach, spleen, and liver are glistening white nodules 0.5 to 1 mm. in diameter and smaller. In the omentum are many, some 4 to 5 mm. in diameter. The omentum is densely matted together and, at the umbilicus, is adherent to the anterior abdominal wall, where there is a firm fibrous nodule the size of a small pea. Strands of connective tissue radiate about this nodule and in its center is soft caseous material. There are also other small nodules on the anterior abdominal wall and in the mesentery.

The bacillary masses in the nodules are surrounded by a large number of polynuclear leukocytes. The development of the connective tissue is fairly well advanced. It forms septa along the periphery of the cells, surrounding the masses of bacteria, as well as concentric fibers about them. About the nodules is a well-developed, but not dense, fibrous capsule.

7. Male, 430 gm., killed May 12. There are numerous nodules on the viscera and on the peritoneal surfaces varying from the very minute to almost pin

point in size. There are some fibrous adhesions between the intestinal loops. The surface of the spleen is free. All of the nodules are white and fibrous.

In the microscopic sections of the nodules are irregular masses of bacteria surrounded by polynuclear leukocytes, about which, in turn, are concentrically arranged epithelioid cells and young connective tissue. There are a few small giant cells in the sections. There is a well-defined fibrous capsule from which septa extend into the interior of the nodules. For the most part the nodule consists of polynuclear leukocytes with small proportion of endothelial cells.

8. Male, 520 gm., killed May 27. There are a few white scars on the inferior surface of the diaphragm. On the liver are several white, pin point nodules, and on the omentum there are a number, 1 to 2 mm. in diameter. There are no nodules on the anterior abdominal wall, mesentery, and intestines. Loops of the intestine are bound together by adhesions in a number of places.

The nodules in the omentum contain a great number of endothelial cells and polynuclear leukocytes, while, scattered at irregular intervals, are small granular masses of bacteria. About the larger bacillary masses are numerous polynuclear leukocytes, bordering which is a zone of endothelial cells. Typical giant cells are irregularly scattered through the nodules. There are also small trabeculae of newly-formed connective tissue.

Summary.—Unabsorbed masses of fat-free bacteria and granulation tissue are present even after 39 days with daily administration of potassium iodid in doses of 0.04 (Animals 1 to 4) and 0.1 gm. (Animals 4 to 8). The nodules are no further in organization than are those of the control animals.

SERIES 4 AND 5. THE INFLUENCE OF POTASSIUM IODID ON THE ABSORPTION OF THE FAT-FREE TUBERCLE BACILLI

April 21. Each guinea-pig in Series 4 and 5 received 0.10 gm. of fat-free tubercle bacilli intraperitoneally and Animals 1 to 4, 0.10 gm. of potassium iodid daily, Animals 5 to 8 serving as controls.

1. Male, 350 gm., found dead May 5. The body is badly decomposed. There are numerous small grayish-white nodules in the mesentery and omentum, and one is present on the liver. No microscopic study.

2. Female, 360 gm., killed May 20. There are a few white scars and several white nodules, about 1 to 2 mm. in diameter, on the inferior surface of the diaphragm. There are about six yellowish-white nodules, 2 to 3 mm. in diameter on the liver, and smaller ones are present on the serous surfaces of the stomach. The omentum is densely matted with hard nodules which have radiating bands of connective tissue about them. There are other white nodules, 3 mm. in diameter and smaller, in the mesentery and on the serosa of the bowel. On the spleen, there are three to four small white nodules, and dense fibrous adhesions bind loops of the bowel together and to the abdominal wall.

The nodules, which are surrounded by a rather thin fibrous capsule, contain bacillary masses about which polynuclear leukocytes are very abundant. These masses are irregular in size and shape. Bordering the zone of leukocytes are epithelioid cells and new connective tissue. There are a few typical giant cells in the granulation tissue, but on the whole the polynuclear leukocytes predominate.

3. Female, 620 gm., killed June 12. On the inferior surface of the diaphragm are two to four small yellowish-white nodules. There are also white areas on the liver, 2 to 3 mm. in diameter. In the omentum are many white nodules, varying in size from a pin head to 3 to 4 mm. in diameter. Fibrous bands bind a few of the intestinal loops together and to the anterior abdominal wall.

Microscopically, the small bacterial masses in the nodules are surrounded by a narrow zone of polynuclear leukocytes, while the larger masses are bordered by a rather wide zone. There are numerous giant cells in the tissue, some of which consist of a narrow margin of protoplasm forming a ring about an engulfed mass of bacteria and with nuclei lying either along the narrow periphery or collected at one end or both poles of the cell. Other giant cells are typical in appearance and contain numerous vesicular nuclei. There are many epithelioid cells and much well-developed connective tissue in the nodules. Septa of connective tissue extend through the nodules, the abundance of vascular fibrous tissue being rather striking.

4. Male, 420 gm., killed June 12. There are two or three small white nodules on the inferior surface of the diaphragm. The surfaces of the liver are free. There are large nodules, 2 to 3 mm. in diameter, in the omentum which bind the intestine together and to the anterior abdominal wall. On the surface of the spleen there are a few white areas about a millimeter and less in diameter.

There are large and small masses of bacteria in the nodules in the peritoneal cavity, the larger areas being surrounded by many polynuclear leukocytes, the smaller in part also, but usually engulfed by giant cells or in the process of being surrounded by a rim of cytoplasm. Surrounding the zone of polynuclears there are many endothelial and young connective-tissue cells which, along the border of the cell masses, have a concentric arrangement. There are many large and small giant cells as well as much vascular connective tissue in these nodules.

5. Male, 340 gm., killed May 20. There are small white scars on the inferior surface of the diaphragm, but no definite nodules. There are adhesions between the left lobe of the liver and the omentum. On the liver are only a few small nodules. The omentum is adherent to the abdominal wall in the region of the spleen and is very dense and nodular. The nodules are yellowish, opaque, and vary in size from a small pea down to a pin head and smaller. There are also adhesions about the spleen and between the intestinal loops.

In the sections prepared from the nodules in the viscera are very many giant cells. There are large irregular masses of bacteria surrounded by numerous polynuclear leukocytes, but around the smaller bacterial masses are rings of protoplasm, which are in all stages of giant-cell formation. There are also many epithelioid and young connective-tissue cells in the nodules. The fibrous capsule about the nodules is firm and dense.

6. Female, 340 gm., killed June 12. There are a few small white scars on the inferior surface of the diaphragm and also a few very small, pale areas on the surfaces of the liver and spleen. These are rather indistinct, and are not sharply differentiated from the surrounding tissues. In the omentum are numerous small white, or yellowish-white, nodules which are adherent to loops of the bowel. There are adhesions between the intestines and the anterior abdominal wall, as well as between the intestinal loops themselves. There are a few white scars on the anterior abdominal wall.

In the microscopic sections of the nodules are masses of bacteria surrounded by a large number of polynuclear leukocytes. For the most part, however, the

small masses of bacteria are engulfed by giant cells, which are abundant, frequently typically formed, but in other places having reached only partial development. Fibrous tissue and epithelioid cells are abundant.

7. Male, 410 gm., killed June 12. There are white scars on the diaphragm which mark the places where the liver is adherent. White scars, three or four in number and about 2 mm. and smaller in diameter, are scattered over the liver. There are dense fibrous adhesions between the liver, intestines, and omentum as well as intestinal adhesions with the abdominal wall. On the spleen and in the mesentery are a few white scars.

In the nodules are many giant cells which have engulfed masses of bacteria, or are in the process of so doing. There are also larger irregular masses of bacteria surrounded by polynuclear leukocytes, which in turn are bordered by fibrous tissue and epithelioid cells. The nodules have a well-developed fibrous capsule. The abundance of giant cells, epithelioid and fibrous connective-tissue cells are prominent features of these nodules.

8. Female, 400 gm., killed June 12. There are a few fibrous adhesions between the liver and the inferior surface of the diaphragm. There are large, scar-like masses in the omentum which are bound to the intestines, forming adhesions between them and the anterior abdominal wall, as well as between the intestinal loops themselves. The spleen is free and of normal appearance.

There are irregular masses of bacteria in the nodules, some of which are surrounded by polynuclear leukocytes, while others are being engulfed by giant cells. Some of the giant cells are typically formed, while others appear as rings of protoplasm about the bacterial masses. Some of the giant cells are large and have twenty to twenty-five clear, oval nuclei irregularly scattered in their cytoplasm, as well as nuclear fragments of ingested polynuclear leukocytes. There is an abundance of fibrous tissue and epithelioid cells in addition to the giant cells.

Summary.—In the controls (Animals 4 to 8) the process of absorption and organization has progressed well, but is not complete after 52 days; absorption and organization are no further advanced in Animals 1 to 4, which received potassium iodid daily.

SERIES 6. THE EFFECT OF POTASSIUM IODID ON LESIONS PRODUCED INTRAMUSCULARLY BY FAT-FREE TUBERCLE BACILLI

Nov. 25, 0.05 gm. of defatted tubercle bacilli, suspended in sterile salt solution, was injected into the large muscles of the left hind leg of two female rabbits of the same weight, one of which received from 0.08 to 0.30 gm. of potassium iodid almost daily from Nov. 25 to Feb. 24.

On Dec. 3, 0.05 mg. of defatted tubercle bacilli was injected into the large muscles of the right hind leg.

On Jan. 1, 0.05 gm. of defatted tubercle bacilli was injected into the large muscles of each front leg.

Several weeks after the injection a large nodule had developed at the site of each inoculation.

Rabbits killed and the nodules removed on Feb. 24.

The nodules removed have the following dimensions:

CONTROL RABBIT

Left hind leg—absent—a little granulation tissue.

Right hind leg—3.5x3.3x1.3 cm.

Left front leg—3.5x3.0x2.0 cm.

Right front leg—3.5x2.7x1.7 cm.

IODID RABBIT

Left hind leg—2.4x1.5x1.2 cm.

Right hind leg—2.5x2.3x1.8 cm.

Left front leg—3.5x3.0x2.5 cm.

Right front leg—3.0x2.5x3.8 cm.

Each nodule has a large caseous center and is well surrounded by a fibrous capsule.

On Dec. 3, 0.05 gm. of defatted tubercle bacilli, suspended in sterile salt solution, was injected into the large muscles of the right hind leg of two rabbits, one of which received 0.08 to 0.30 gm. of potassium iodid almost daily from Dec. 3 to March 12.

Jan. 24, injection repeated, this time into the large muscle of each front leg.

Several weeks after the injection of the bacilli a large nodule developed at the site of each inoculation.

The rabbits were killed and lesions removed on March 18.

The nodules removed have the following dimensions:

CONTROL RABBIT

Right hind leg—1.2x2.0x2.2 cm.

Right front leg—3.5x2.0x2.0 cm.

Left front leg—about the size of a small pea, probably because of faulty injection or discharge upon the surface.

IODID RABBIT

Right hind leg—1.5x2.0x3.0 cm.

Right front leg—4.0x2.5x2.5 cm.

Left front leg—4.0x3.0x1.5 cm.

All of these nodules have a caseous center and are surrounded by a firm fibrous capsule.

There is practically no difference in the rate of absorption in these animals, even tho the iodid rabbits received iodid over a considerable period of time.

SERIES 7. THE EFFECT OF IODIZED FAT-FREE TUBERCLE BACILLI WHEN INJECTED
INTRAMUSCULARLY IN GUINEA-PIGS

On Jan. 6, injected 0.05 gm. of defatted tubercle bacilli into the muscles of the hind legs of two guinea-pigs of same weight. The bacilli injected into the right leg were treated for fifteen minutes with an iodine solution (I=1 gm.; KI = 2 gm.; water = 400 c.c.), sedimented by centrifuging, and suspended in sterile salt solution. The bacilli injected into the left leg were untreated.

The animals were killed on Feb. 11 and the nodules in the leg muscles removed. The dimensions of the nodules were as follows:

Guinea-pig 1. Right—2.0x1.5x1.8 cm.; left—2.0x1.3x1.0 cm.

Guinea-pig 2. Right—2.5x2.5x2.0 cm.; left—2.5x2.5x1.8 cm.

Summary—No difference is noted in the size of the nodules produced by either the iodized or the non-iodized tubercle bacilli.

SERIES 8. THE INFLUENCE OF IODIN, IODOFORM AND IODID ON THE ABSORPTION OF AGAR

Controls.—On May 7, 1.5 c.c. of 2 percent sterile agar jelly was injected intraperitoneally in two guinea-pigs.

1. Found dead May 12. On the surface of the spleen are white areas 3 to 5 mm. in diameter. There are only suggestions of nodules in the omentum.

The nodules on the spleen consist of irregular masses of agar broken up by strands of fibrin in the meshes of which there are numerous polynuclear leukocytes and endothelial cells. Along the margin of the nodules are numerous endothelial cells, some of which have fragmented nuclei and vacuolated cytoplasm. In only a few places are giant cells beginning to form.

2. Killed May 19. There are two to four nodules of agar, 2 mm. and smaller in diameter, in the omentum. On the surface of the spleen are two or three nodules, about 1 mm. in diameter. There are a few fibrous adhesions between loops of the bowel.

In the lymphoid tissue removed from the mesentery are a few nodules which simulate a tubercle in their microscopic appearance, differing, however, in that their centers consist of a large mass of polynucleated leukocytes which form an area approximately one-fourth the diameter of the nodules. Surrounding this are concentrically arranged fibroblastic and endothelial cells. There are budding capillaries in the fibroblastic tissue as well as a few clearly defined giant cells. These small areas probably represent almost completely absorbed and organized masses of agar. On the surface of the lymph gland are small masses of agar broken up into particles by fibrin, endothelial cells, and young connective-tissue cells.

3. Killed May 28. There is a firm white nodule 5 mm. in diameter in the region of the spleen where the bowel is also adherent. There are a few pearly nodules, 1 mm. in diameter, in the mesentery, and in the omentum there is one about 2 mm. in diameter with adhesions binding it to the viscera. There is another slightly larger nodule in the intestinal loops where there are also firm fibrous adhesions. On the surfaces of the liver and spleen are a few white nodules 1 mm. in diameter.

The nodules in the viscera contain numerous small masses of agar invaded and broken up into small bits by connective tissue and endothelial cells. There are numerous giant cells in this tissue and the process of organization is well advanced. The giant cells on the whole are small, containing, on an average, from four to eight nuclei.

Iodized Agar.—In these experiments 2 c.c. of iodine solution ($I=1$ gm.; $KI=2$ gm.; water=400 c.c.) were added to 7 c.c. of 2 percent agar agar and 1.5 to 2.0 c.c. of the mixture injected intraperitoneally May 7.

1. Found dead May 14. There are three or four white nodules, about 3 mm. in diameter, on the spleen. No other changes were observed.

The capsule of the spleen beneath the nodules is thickened by new connective tissue, and, immediately beneath it, the tissues are hyperemic. The nodules themselves consist of masses of agar which are being broken up into smaller bits by connective tissue and endothelial cells. The process of organization on the whole is only fairly well advanced.

2. Killed May 19. The mesenteric lymph glands are enlarged, and in the mesentery there are two light-brown, rather firm masses approximately $8 \times 5 \times 3$ mm. in their greatest dimensions. On the surface of the liver and spleen are

a few pin-head and smaller sized pearly-gray nodules. There are two or three nodules about 1 mm. in diameter in the omentum.

The nodules on the surface of the spleen consist chiefly of agar which is broken up by fibrin strands in which there are also a few polynuclear leukocytes, endothelial, and connective-tissue cells. There is no very marked cellular reaction excepting along the periphery where the connective-tissue cells are relatively abundant.

3. Killed May 28. There are many pearly white nodules, 1 mm. and slightly larger in diameter, in the omentum and about the spleen. There are also numerous small fibrotic masses in the mesentery.

The splenic capsule is thickened in areas which contain small masses of agar rather completely infiltrated with endothelial cells and polynuclear leukocytes, as well as vascular connective tissue. On the surface of the splenic capsule are irregular masses of agar which are being invaded by connective tissue and endothelial cells, while in certain places the organization of the agar is fairly well advanced.

Iodoform Injections.—On May 7, 1.5 c.c. of 2 percent agar agar were injected intraperitoneally and 0.5 c.c. of a 10 percent iodoform-glycerol suspension was injected deep into the muscles of the right hind leg.

1. Killed May 19. There is one white nodule, about 2 mm. in diameter in the omentum, and there are a few smaller ones on the serous surface of the gall bladder. At the site of the inoculation there are a few adhesions with the bowel. The surfaces of the spleen and liver are free.

2. Killed May 19. There are firm adhesions between parietal peritoneum and a large mass in the omentum about $15 \times 7 \times 2$ mm. There are also numerous white nodules in the omentum, about 1.5 mm. in diameter. The mesenteric lymph glands are greatly enlarged. Iodoform is present in the tissues at the site of its injection.

The nodules in the omentum contain irregular masses of agar which, along their borders, are being infiltrated by cells chiefly of the endothelial type, but also a few polynuclear leukocytes. There the agar is broken up into smaller masses which are being engulfed by giant cells. The giant cells are numerous and in all stages of development from narrow rims of cytoplasm about the agar masses to others which simulate closely typical Langhans giant cells. The tissue, aside from that already mentioned, consists chiefly of newly-formed granulation tissue.

3. Killed May 27. There are a number of white nodules in the omentum, about 1 mm. in diameter, and also a few fibrous adhesions between the intestinal loops. Iodoform is present in the tissues at the site of the injection.

The masses of agar in the omentum are well along in the process of organization. There are a few giant cells in the granulation tissue.

Potassium Iodid.—On May 27, 1.5 c.c. of 2 percent agar agar were injected intraperitoneally and thereafter 0.04 to 0.1 gm. of potassium iodid daily.

1. Found dead May 12. There are a few nodules, 2 to 5 mm. in diameter, on the anterior abdominal wall and also a few miliary sized nodules on the omentum and on the surfaces of the spleen. There are about three to six small, thin flakes of agar free in the peritoneal cavity.

The nodules present on the viscera contain agar which is in the early stage of organization, being invaded partly by endothelial, connective-tissue cells and a few polynuclear leukocytes.

2. Found dead May 19. In the omentum there are three to six white nodules, about 1 mm. in diameter. The liver surfaces are covered by many white areas pin-head and under in size. There are also a few on the spleen. At one place, there are dense fibrous adhesions between several loops of the bowel. There is a thin flake of agar, 1 mm. in diameter, floating freely in the peritoneal cavity.

3. Killed May 27. There are a few white nodules and scars, a millimeter or so in diameter, in the omentum and on the surface of the liver. There are a number of fibrous adhesions between the loops of the bowel.

The small nodules on the liver surface are fairly well organized and contain small masses of agar infiltrated by connective tissue and endothelial cells in which are a few small giant cells with only four to six nuclei.

In the following experiments, 1.5 to 2.0 c.c. of glycerol agar (agar agar 1.5 percent; glycerol 1 percent) were injected intraperitoneally on Feb. 26 and also into the large muscle of each hind leg. Beginning Feb. 26 and thereafter 0.10 gm. of potassium iodid was given daily to Pigs 1 and 2.

1. Found dead March 13. There are only three or four thin flakes of agar agar, 2 by 3.5 mm., floating in the peritoneal cavity. There are no nodules in the mesentery, omentum, or leg muscles.

2. Found dead March 10. There are several small masses of agar 1 to 2 mm. in diameter on the surface of the liver. On the spleen there are about six or eight similar nodules 1 to 3 mm. in diameter. A small flake of agar 2 x 2 x 5 mm. floats in the peritoneal cavity. In the leg muscles there is only a small granulation area about 2 to 3 mm. in diameter.

4. Killed March 13. There are only a few small masses of agar in the omentum and a few thin flakes, about 1 mm. in diameter, in the leg muscles.

5. Killed March 13. A mass of agar, 1 cm. in diameter, is present in the omentum opposite the left seminal vesicle. There are also numerous small nodules in the omentum and mesentery. There are a few small flakes in the leg muscle.

In the following experiments, 3.0 c.c. of 2 percent agar agar was injected intraperitoneally on March 3, and 0.04 to 0.10 gm. of potassium iodid was given daily to Pigs 3 and 4.

1. Killed April 20. In the omentum and mesentery, on the liver, and spleen are numerous nodules of agar, varying in size from a pin point to one in the mesentery 10 x 8 x 10 mm.

2. Killed April 20. There is a large number of pin-head and smaller sized agar nodules in the mesentery, omentum, on the spleen and liver.

3. Killed April 20. In the peritoneal cavity, there is a single white mass the size of an English walnut which lies in close relation with the cecum and the small bowel. The center of this mass contains soft purulent material. There are no other nodules, the entire agar injection apparently having localized in this spot and subsequently became infected.

4. Killed April 20. There are numerous pin-head and smaller sized nodules of agar in the mesentery and omentum, on the inferior surface of the diaphragm, and on the anterior wall of the abdomen. The connective tissue reaction about these nodules seems no greater than in the controls.

Summary.—The results do not indicate that iodine, iodoform, or potassium iodid hastened the absorption of agar.

DISCUSSION

The microscopic studies of the lesions, produced in guinea-pigs by fat-free tubercle bacilli and agar jelly, disclose little difference in the intensity of the cellular reactions about the nodules whether the animals are given iodids or remain uniodized. When fat-free bacilli are used, the reaction about the bacillary masses is chiefly a polynuclear leukocytic infiltration, the abundance of the cells in a given area depending much on the size of the bacillary masses and the length of time the bacilli have been incorporated in the tissues. Endothelial and connective tissue cells gradually appear about the aggregations and, with the progressive fibrosis of the nodules, there is a gradual diminution in the leukocytes. The final destruction of the bacilli is accomplished, apparently, by endothelial phagocytes, which fuse about bacterial masses to form giant cells.

The cellular reaction about the agar jelly is, on the whole, not as active, or as marked, as it is about the masses of bacteria. There are not as many polynuclear leukocytes taking part in the organization, while the endothelial cells are relatively more abundant.

Cantacuzene reported the complete disappearance of tuberculous formations produced by 0.01 gm. of fat-free bacilli in five or six days if the guinea-pig received 0.1 gm. of potassium iodid daily, as contrasted with the lesions in control animals not receiving potassium iodid, which persisted one or two months. Among the experiments recorded here, the same conditions are repeated, but, even after forty-two days, there are well-defined, unabsorbed bacillary masses and tuberculous granulation tissue. The process of organization and absorption in the lesions has progressed no further than in the control animals. Neither do the caseous nodules produced in the leg muscles of iodized rabbits have any striking difference in size, or in degree of organization, from those in the control rabbits, even tho large doses of potassium iodid were given over a period more than three months.

The apparently increased fibrous tissue reaction about the masses of fat-free tubercle bacilli which had been treated with iodine solution, prior to their intraperitoneal inoculation, demands some explanation. Carefully analyzed, this is not difficult to understand. Heinz² injected aseptically an iodine solution into the pleural and peritoneal cavities of rabbits, and observed a fibrinous exudative inflammation in both. The masses of fat-free tubercle bacilli have a dark brown color after being removed from the iodine solution. The brown color gradually dis-

2. *Virch. Arch.*, 1899, 155, p. 44.

appears from the bacteria as the iodine diffuses into the suspending fluid if this is changed, indicating that the combination of the iodine with the bacterial protein is, at least partially physical, rather than chemical. If bacterial masses are injected into the peritoneal cavity before the iodine has diffused into the suspending liquid, but subsequently does so in the tissues, there will appear a fibrinous inflammation about the bacilli, which organizes readily and makes the appearance of a greater fibrous tissue reaction about the nodules. In the interior of these nodules, the masses of bacteria are in no further stage of absorption than are the masses in the peritoneal cavities of guinea-pigs receiving no iodine. Such guinea-pigs, as received iodized and non-iodized bacilli in opposite leg muscles, developed nodules which progressed no differently either in the extent of their development or in the rate of their absorption.

While the experimental evidence in connection with the fat-free tubercle bacilli is perhaps more extensive, that with the agar jelly is no less convincing. Here too, there is practically no difference in the degree of agar absorption and organization whether the guinea-pigs are iodized or non-iodized.

These observations have an important bearing on the prevalent idea that iodides facilitate the absorption of granulation tissue and necrotic areas. The value of iodides in tertiary syphilis cannot be denied, but here also their therapeutic action is not understood. By means of experiments, the effects of iodine and its compounds may be defined more closely and, with this knowledge, a more precise therapeutic application in various retrogressive processes becomes possible.

CONCLUSIONS

The use of iodine and iodides, in facilitating the absorption of necrotic material and organization of tuberculous and other granulation tissues, has no experimental proof.

The daily administration of potassium iodide does not hasten the removal of foreign substances, like tubercle bacilli, by stimulating the phagocytic properties of the endothelial cells. The presence of free iodine in such areas may influence the process only by favoring an inflammatory reaction, if the amount of iodine is sufficient, but has no effect in promoting absorption.

Iodized fat-free tubercle bacilli are absorbed no faster than are the non-iodized.